

Factors Affecting Germination and Establishment Success of an Endemic Cactus of the Chihuahuan Desert

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Factors affecting germination and establishment success of an endemic cactus of the Chihuahuan Desert

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Abstract Seeds and seedlings are the most critical stages of cacti life cycles. From the thousands of seeds produced in a reproductive season, only a small fraction successfully germinate, the rest are lost to predation, go dormant and remain viable in the seed bank or lose viability. These early stages often depend on facilitation by nurse plants for germination and seedling recruitment. We aim to prescribe actions for the conservation of *Cephalocereus polylophus* by improving recruitment in the population. The viability of seeds with different storage times was evaluated as an indicator of their potential to form a short-term seed bank. Through the analysis of seed germination and seedling survival under the canopy of two nurse plant species and open areas, we assessed the importance of facilitation for recruitment. A predator exclusion experiment evaluated the intensity of herbivory on seeds and seedlings of different ages. Seeds had germination rates above 90% under laboratory conditions, even after two years of storage. Seed germination was only

registered under one of the two nurses and after two years, up to 4 % of the seedlings planted under both nurse plants survived and protection against herbivores increased seedlings survival. Considering that facilitation and age are crucial for seedling survival of *C. polylophus*, future conservation programs should include the protection of plant communities and the introduction of seedlings instead of seeds.

Keywords Facilitation · seedling recruitment · seed germination · *Cephalocereus polylophus* · seed and seedling limitation · Cactaceae

1 Introduction

Cacti are among the most ubiquitous plant families of North and South American hot deserts (Guerrero et al., 2019). Many cacti species release large numbers of seeds to their populations on an annual basis, but most are rapidly removed by birds, rodents, and ants before having the chance to germinate (Sosa and Fleming, 2002; García-Chávez et al., 2010; Holland and Molina-Freaner, 2012). The small fraction of seeds that escape granivory might remain viable in or on the soil for months to several years (Rojas-Aréchiga and Vázquez-Yanes, 2000; Holland and Molina-Freaner, 2012), but not much is known about seed predation rates, the formation of soil seed banks and multi-year dormancy in the Cactaceae (Holland and Molina-Freaner, 2012).

Seedlings are one of the central components of plant population dynamics (Harper, 1974). The recruitment of seedlings, understood as the process by which new individuals are added to a population, includes seed germination, seedling survival, and seedling growth (Eriksson and Ehrlén, 2008). Seedlings play a central role in the life cycles of many plant species from arid environments (Valiente-Banuet and Ezcurra, 1991; Godínez-Álvarez et al., 2003; Pierson et al., 2013); however, plant populations in these

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52 areas can be severely seedling limited (Rees, 1994) by bi-
 53 otic and abiotic factors, such as high temperatures (Nobel,
 54 1984; Suzán-Azpiri and Sosa, 2006; Miranda-Jácome et al.,
 55 2013), low and unpredictable water availability (Holland
 56 and Molina-Freaner, 2012), and strong predation pressures
 57 (García-Chávez et al., 2010; Holland and Molina-Freaner,
 58 2012), all of which negatively impact seed and seedling sur-
 59 vival (Bowers, 1997; Pimienta-Barrios et al., 2002; Godínez-
 60 Álvarez et al., 2003; Rojas-Sandoval and Meléndez-Ackerman,
 61 2012; Pierson et al., 2013).

62 Plant facilitation provides a positive interaction for seed-
 63 lings, especially during the recruitment, in which benefac-
 64 tor species (nurse plants) enhance the micro-habitat condi-
 65 tions beneath their canopy for the facilitated plants (Flores-
 66 Martínez et al., 2009; Verdú et al., 2010; Aranda-Pineda et al.,
 67 2021). The importance of this type of association has been
 68 reported for several cacti species, which benefit from the
 69 interaction with shrubs from the families Fabaceae, Aster-
 70 aceae, Mimosaceae, and Asparagaceae (Nobel, 1984; Franco
 71 and Nobel, 1989; Valiente-Banuet and Ezcurra, 1991; Man-
 72 dujano et al., 1998, 2001; Rojas-Sandoval and Meléndez-
 73 Ackerman, 2012). The micro-habitat created beneath the
 74 canopies of nurse plants has been shown to protect seedlings
 75 by reducing the total daily photosynthetically active radia-
 76 tion (PAR) (Franco and Nobel, 1989), decrease the maxi-
 77 mum soil surface temperature (Nobel, 1980; Franco and No-
 78 bel, 1989; Munguía-Rosas and Sosa, 2008; Miranda-Jácome
 79 et al., 2013), reduce evapotranspiration (Valiente-Banuet
 80 et al., 1991), improve soil properties (Munguía-Rosas and
 81 Sosa, 2008; Montesinos-Navarro et al., 2016) and protect
 82 seeds and seedlings from predation (Mcauliffe, 1984; Valiente-
 83 Banuet and Ezcurra, 1991; Sosa and Fleming, 2002; Holland
 84 and Molina-Freaner, 2012).

85 The conservation of many cacti species would benefit
 86 from a clear understanding of the biotic and abiotic factors
 87 that play a role in processes that constrain population dy-
 88 namics (Ureta and Martorell, 2009). Using *Cephalocereus*
 89 *polylophus* as a study species, we aim to answer the follow-
 90 ing questions: what is the short-term seed viability?, how
 91 important is facilitation for seed germination and seedling
 92 survival?, how intense is seedling predation and which is the
 93 most vulnerable seedling age?

94 2 Materials and methods

95 2.1 Study species

96 *Cephalocereus polylophus* (DC.) Britton & Rose (recently
 97 transferred from *Neobuxbaumia polylopha* (Tapia et al., 2017))
 98 is a columnar cactus that occurs naturally in canyon regions
 99 covered with deciduous forest and calcareous soils. It is dis-
 100 tributed in an area of approximately 6,000 km² constrained to

101 six isolated locations in central Mexico (IUCN, 2019). Pop-
 102 ulations are dispersed but some have high densities (mean
 103 \pm SD 0.67 ind/m² \pm 0.28) (Arroyo-Cosultchi et al., 2016).
 104 The flowering season is between May and July, flowers are
 105 hermaphroditic, large (length 4.63 \pm 0.08 cm) with a dark
 106 pink perianth, anthesis is nocturnal and lasts for one and
 107 rarely two nights (Anderson, 2001; Arroyo-Cosultchi et al.,
 108 2010). Flowers are nectariferous and are visited by bats and
 109 hummingbirds (Cornejo-Latorre et al., 2011). The number
 110 of seeds per fruit is 976 \pm 80.42 (mean \pm S.E.) (Arroyo-
 111 Cosultchi et al., 2016). Seed length and width is 2.68 \pm 0.04
 112 \times 1.85 \pm 0.02 mm (mean \pm S.E.) (Arroyo-Cosultchi et al.,
 113 2007), and are dispersed by bats, birds, and ants during July
 114 and August (Arroyo-Cosultchi et al., 2016).

115 2.2 Study area

116 The study was carried out in the Barranca de Metztitlán Bio-
 117 sphere Reserve (RBM), in the vicinities of the town of San
 118 Miguel Almolón (20° 43' 32.8" N - 98° 54' 56.9" W), in
 119 the state of Hidalgo, Mexico. RBM is considered one of the
 120 most outstanding cactus regions in the country, 70 species
 121 are found in the region, 11.42 % of which are endemic and
 122 15 % are under some category of risk (Sánchez-Mejorada,
 123 1978; CONANP, 2003). The climate is dry, semi-warm with
 124 summer rains (BS0hw), mean annual temperature is 20.7 °C
 125 (min-max 14.1 - 27.3 °C), mean annual precipitation is 411.4
 126 mm (min-max 184.1 - 810.1 mm) with near 85 % of annual
 127 precipitation occurring during summer and fall (CONAGUA,
 128 Meteorological Station San Cristobal, Metztitlán; 20° 38'29"
 129 N - 98° 49' 42.96" W). During the study period (June 2014 –
 130 June 2016), mean annual rainfall was 544.66 mm with peaks
 131 occurring in September. The dominant vegetation type is
 132 xerophytic scrub (CONANP, 2003) and the vegetation is clas-
 133 sified as deciduous forests and microphyllous scrubs, domi-
 134 nated by *Croton mazapensis* Lundell, *Hoverdenia speciosa*
 135 Nees, as well as shrubby legumes (Herce et al., 2013) and the
 136 columnar cactus *Cephalocereus senilis* (Haw.) Pfeiff., Allg,
 137 and *Isolatocereus dumortieri* (Scheidw.) Backeb. (Cruz and
 138 Pavón, 2013).

139 2.3 Seed collection

140 Three mature fruits from 30 different *C. polylophus* indi-
 141 viduals were collected during the 2012, 2013 and 2014 re-
 142 productive seasons. Immediately after collecting the fruits,
 143 seeds were cleaned, dried and stored in labeled paper bags at
 144 room temperature to avoid fungal infestation. A sub-sample
 145 of seeds from its respective cohort was mixed to have a rep-
 146 resentative variability within fruits an individual plants.

147	2.4 Seed limitation		
148	2.4.1 Seed viability		
149	Seed viability of <i>C. polylophus</i> over time was assessed in		
150	September 2014. Seeds from the 2012, 2013, and 2014 co-		
151	horts were germinated under laboratory conditions. Experi-		
152	mental units consisted of a Petri dish with 1% bacteriolog-		
153	ical agar in which ten seeds from one of the three cohorts		
154	were sown. Each seed cohort had thirty replicates ($n = 90$		
155	experimental units). Experimental units were placed in an		
156	environmental chamber under controlled temperature and		
157	photoperiod (Lab-Line Biotronette 845, 28-30 °C and 12 h		
158	photoperiod). Germination was registered daily for 30 days.		
159	In order to assess differences in seedling development by		
160	cohort, the length and diameter of every germinated seedling		
161	were measured with a digital caliper to the nearest 0.01 mm		
162	after 30 days.		
163	2.4.2 Seed germination under different PAR intensities		
164	Light is one of the most important environmental factors to		
165	which cacti seed germination is regulated (Rojas-Aréchiga		
166	et al., 2013). <i>Cephalocereus polylophus</i> is neutral photo-		
167	blastic (Rojas-Aréchiga, 2014), but it is unknown to which		
168	degree the germination rate is affected by different PAR		
169	intensities. As an experimental simulation of the low PAR		
170	intensities expected under the canopy of shrubs, we assessed		
171	how germination is affected by different low PAR intensities		
172	in laboratory conditions. The maximum PAR capacity of the		
173	environmental chamber was equivalent to 31 % of the mean		
174	PAR registered in open areas in habitat. Fresh seeds collected		
175	in the previous reproductive season (July 2013) were subject		
176	to four different light treatments inside an environmental		
177	chamber (Lab Line Biotronete 845), calibrated to perform		
178	at a maximum photosynthetically active radiation (PAR) of		
179	$394 \mu\text{mol}^{-2} \text{s}^{-1}$, 28-30 °C and 12 h photoperiod. The full		
180	radiation of the environmental chamber was used as the 100		
181	% PAR treatment and greenhouse shade cloth was used to		
182	simulate lower PAR intensities as follows: 45 % ($182 \mu\text{mol}$		
183	$\text{m}^{-2} \text{s}^{-1}$), 25 % ($103 \mu\text{mol} \text{m}^{-2} \text{s}^{-1}$) and 10 % ($38 \mu\text{mol}$		
184	$\text{m}^{-2} \text{s}^{-1}$); PAR was measured with a Li250A LI-COR sen-		
185	sensor. Every treatment had 30 replicates, each with 20 seeds		
186	sown in Petri dishes with 1 % bacteriological agar, for a total		
187	of 120 experimental units. Germination was registered daily		
188	for 30 days. In order to assess the effect of different PAR		
189	intensities on seedling development, the length and diameter		
190	of 100 seedlings from every treatment were measured with		
191	a digital vernier caliper to the nearest 0.01 mm at the end of		
192	the experiment.		
	2.4.3 Seed predation		193
	Seed predation and removal was studied in natural conditions.		194
	We did not test the experiment in open areas since our exper-		195
	imental units could not be fixed to the bare rock present in		196
	these areas. A randomized block design with two conditions		197
	(protected and exposed seeds), ten replicates and three experi-		198
	mental units per replicate was used. The protected seeds were		199
	placed inside small wire boxes (10 cm^3 and 1 mm aperture		200
	wire mesh), while the exposed seed were left unprotected. To		201
	keep track of the seeds, our experimental units consisted of		202
	thin plastic mesh strips to which ten seeds were glued with		203
	a very small drop of liquid silicone to one of its sides. A		204
	previous experiment with this method showed that the liquid		205
	silicone did not interfere with germination (Ortiz-Martínez		206
	et al. in review). Thirty days after sowing, the number of		207
	remaining seeds in each one of the experimental units was		208
	counted ($n = 60$ experimental units). Lost seeds were consid-		209
	ered as removed whereas empty seed coats were considered		210
	as predated.		211
	2.4.4 Nurse effect on seed germination		212
	Facilitation effects on germination and predation of <i>C. poly-</i>		213
	<i>lophus</i> seeds in its habitat were evaluated during the 2014		214
	rainy season (August). To assess if deciduous shrubs facili-		215
	tate <i>C. polylophus</i> germination, we placed seeds beneath the		216
	canopy of twenty-three individuals of <i>Croton mazapensis</i> and		217
	<i>Hoverdenia speciosa</i> as well as in open areas. PAR beneath		218
	the canopy of each of the twenty-three individuals of each		219
	shrub species and open areas was measure (Li250A LI-COR		220
	sensor). To keep track of the seeds, our experimental units		221
	consisted of thin plastic mesh strips to which ten seeds were		222
	glued with liquid silicone to one of its sides. Two experimen-		223
	tal units were placed beneath each one of the twenty-three		224
	individuals of each nurse species and in twenty-three open ar-		225
	ees ($n = 69$ experimental units). The experimental units were		226
	placed with the side containing the seeds facing the soil, and		227
	were then fixed with nails to avoid removal by wind or water.		228
	The number of germinated, removed and predated seeds was		229
	registered 30 days after sowing. Lost seeds were counted		230
	as removed whereas empty seed coats were considered as		231
	predated.		232
	2.5 Seedling limitation		233
	2.5.1 Nurse effect on seedling survival		234
	The importance of nurse plants for seedling establishment		235
	was evaluated from 2014 to 2016. We tested the survival of <i>C.</i>		236
	<i>polylophus</i> seedlings under the canopy of <i>C. mazapensis</i> , <i>H.</i>		237
	<i>speciosa</i> and in open spaces. Seeds were germinated in labo-		238
	ratory conditions ten months before they were transplanted		239

to habitat. A month before the beginning of the experiment, seedlings were hardened for a month in a greenhouse (Ortiz-Martínez et al., in review). Experimental units consisted of 12 cm biodegradable jiffy pots filled with soil from the study site and ten 10-month-old seedlings transplanted within. One experimental unit was transplanted beneath the canopy of each of the twenty-three individuals of the two species of nurse shrubs and 23 open areas ($n = 69$ experimental units). Seedling survival was recorded daily for the first 12 days and then 1, 2, 5, 12, and 24 months after their introduction to habitat.

2.5.2 Seedling predation

To test whether predation rates were affected by seedling age, we tested the survival of seedlings of different age in partially shaded sites during a two-year period (2014- 2016). We did not test the experiment in open spaces since we expected high mortality rate in these sites((see results of the nurse effect, all seedlings in open areas die during the first twelve days). Seeds were germinated under laboratory conditions five, ten, and twelve months before the seedlings were transplanted to habitat. A randomized block design with six conditions and ten replications was used. The six conditions were: protected twelve month-old, protected ten month-old, protected five month-old, exposed twelve month old, exposed ten month-old, and exposed five month-old seedlings. The experimental units consisted of jiffy pots with ten seedlings from every age group. The experimental units of the protected condition were caged inside small wire mesh boxes (10 cm³, 1 mm mesh aperture), while the ones in the exposed condition were left unprotected ($n = 60$ experimental units). Seedling survival was recorded daily for the first 12 days and then 1, 2, 5, 12, and 24 months after their introduction to habitat.

2.6 Statistical analysis

A general lineal model (GLM) with a binomial error distribution was used to determine differences in the germination experiments. Seed predation was analysed with GLM with a Poisson error distribution. ANOVA followed by Tukey *post hoc* tests (Crawley, 1990) was used to evaluate differences in the length and diameter of seedlings from different cohort and under different light treatment. A correlation analysis was performed to detect a relation between the length and diameter of the seedlings. ANOVA followed by Tukey tests was used to assess differences of PAR beneath the canopy of the nurse plants and open spaces. Survival curves of seedlings from the nurse effect and the age and exclusion experiments were analyzed using Kaplan-Meier (Kaplan and Meier, 1958), the statistical differences among treatments were tested using the Log-Rank test. All data analysis were done using R software v.4.0.2 (R Development Core Team, 2020). GLM

and ANOVA were done using the R Stats Package v.4.0.2 (R Development Core Team, 2020); survival analysis were performed with the survival and survminer packages (Therneau, 2020; Kassambara et al., 2020).

3 Results

3.1 Seed limitation

3.2 Seed viability

Seed germination did not differ between age cohorts ($\chi^2 = 0.3496$, $Df = 2$, $p = 0.8396$), in fact germination of the seeds from all cohorts was remarkably high ($95.7 \pm 5.9\%$). In contrast, seedling length ($F_{2,826} = 455$, $p < 0.001$) and diameter ($F_{2,826} = 235$, $p < 0.001$) were statistically different between cohorts (Table 1). Seedlings from the 2014 cohort were the longest but also the smallest in diameter. Correlation between seedling length and diameter was weak ($r = -0.160$, $p < 0.01$).

3.3 Seed germination under different PAR intensities

There were no significant differences in germination under different Photosynthetically Active Radiation (PAR) ($\chi^2 = 3.12$, $Df = 3$, $p = 0.3732$), germination rate of all treatments was $90 \pm 7.02\%$. Differences were found in the length of seedlings ($F_{3,144} = 123.29$, $p < 0.05$), such that seedlings were longest at lower PAR (Figure 1 a). Seedling diameter was also different ($F_{3,144} = 69.95$, $p < 0.05$), but not between the seedlings exposed to the lowest and highest PAR (Figure 1 b). Correlation between seedling length and diameter was weak ($r = 0.279$, $p < 0.05$).

3.4 Seed predation

No significant differences were observed in the removal of protected and exposed seeds ($\chi^2 = 0.3712$, $Df = 1$, $p = 0.542$). Cages that offered protection only prevented the removal of seeds by vertebrate granivores but did not prevent granivory by the small granivores such as ants of *Campanotus planatus* Roger were seen to be the main granivore of *C. polylophus* seeds. In the protected blocks, $66.6 \pm 30.3\%$ of the seeds were removed, whereas $62.66 \pm 37.0\%$ of the exposed seeds were removed. Seeds were equally predated in the protected and exposed blocks (8%).

3.5 Nurse effect on seed germination

ANOVA showed that the differences in PAR beneath the canopy of the shrubs and open areas were marginally non-significant ($F_{3,144} = 3.14$, $Df=2$, $p = 0.052$). A *post hoc*

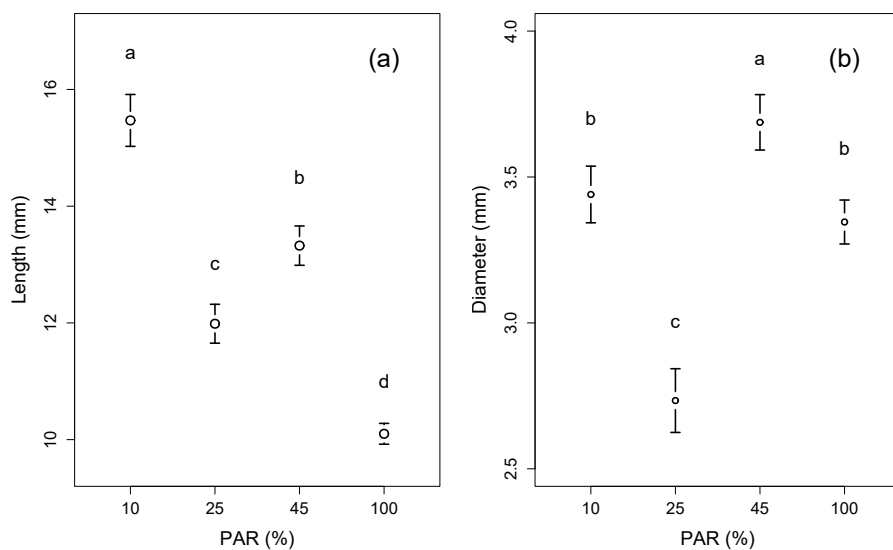


Fig. 1 Length (a) and diameter (b) (Mean± confidence interval 95 %) of *Cephalocereus polylophus* seedlings germinated under different Photosynthetically Active Radiation (PAR) intensities. Different letters indicate differences among PAR treatments for the response variable ($p < 0.05$).

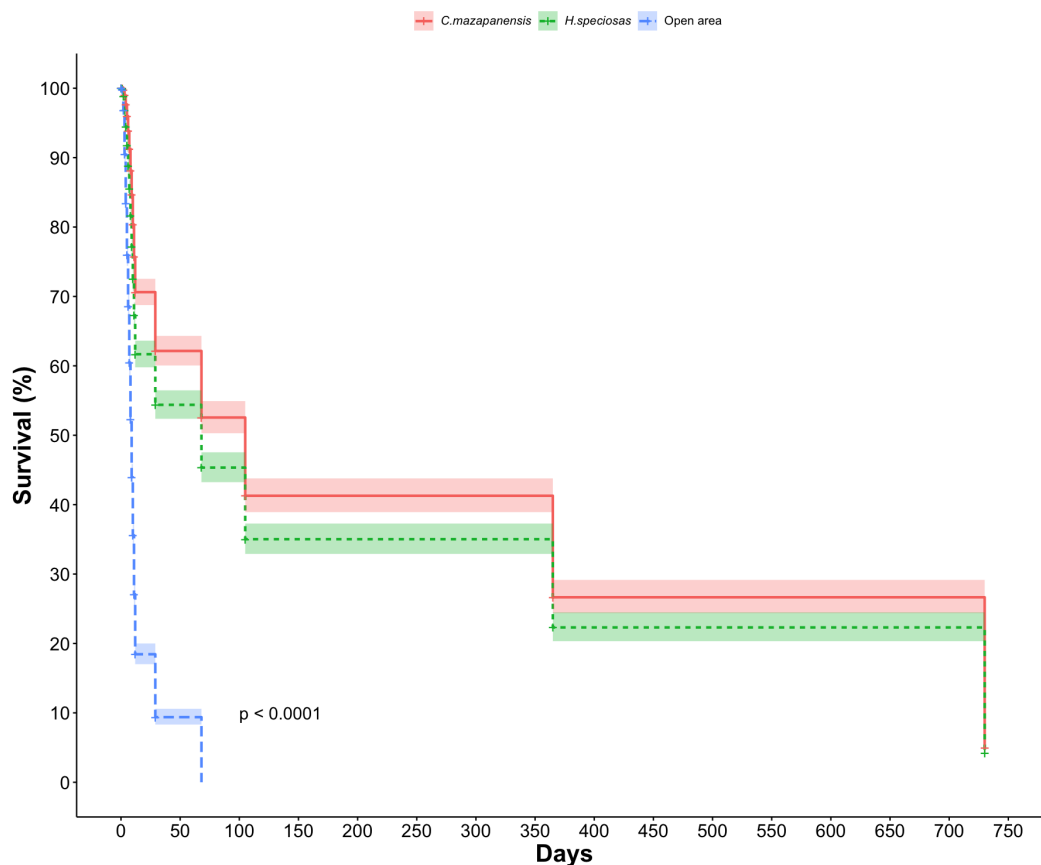


Fig. 2 Survival of 10 month-old *Cephalocereus polylophus* seedlings transplanted under *Croton mazapansensis*, *Hoverdenia speciosas* and open areas during a two year period (August 2014 - August 2016) at Barranca de Metztitán Biosphere Reserve, Hidalgo, Mexico using Kaplan-Meier's method. Confidence intervals at 95 % (width of the curve), $n_{initial}=230$ seedlings per condition.

Table 1 Germination rate, length and diameter (mean \pm standard error) of 30 days old *Cephalocereus polylophus* seedlings from different cohort seeds ($n = 830$ seedlings). Different letters indicate statistically clear differences ($p < 0.01$).

Year of collection	Germination rate (%)	Length (mm)	Diameter (mm)
2012	93.66 a	8.15 \pm 0.05 c	3.92 \pm 0.03 b
2013	98.33 a	8.48 \pm 0.05 b	4.18 \pm 0.03 a
2014	95.66 a	10.37 \pm 0.07 a	3.54 \pm 0.02 c

Tukey test indicated that PAR beneath the canopy of *Croton mazapensis* ($894.85 \pm 512.30 \mu\text{mol}^{-2} \text{s}^{-1}$) was significantly lower than in open areas ($1725.83 \pm 247.11 \mu\text{mol}^{-2} \text{s}^{-1}$) ($p = 0.044$), and no significant differences were found between *Hoverdenia speciosa* ($1054.37 \pm 597.31 \mu\text{mol}^{-2} \text{s}^{-1}$) and open areas ($p = 0.123$). Only the species with highest PAR amelioration, *C. mazapensis*, facilitated germination of *C. polylophus* ($2.39 \pm 9.47 \%$). No germination was recorded under the canopy of *H. speciosa* or in open spaces.

Seed removal in open areas ($76.9 \pm 28.66 \%$) was higher than under the canopy of *H. speciosa* ($65.0 \pm 33.99 \%$) ($\chi^2 = 4.300$, $p = 0.038$) and not statistically different than under *C. mazapensis* ($67.6 \pm 34.9 \%$) ($\chi^2 = 2.630$, $p = 0.104$). Seed predation was also lower beneath the canopy of *H. speciosa* ($5.68 \pm 9.49 \%$) than in open areas ($9.76 \pm 12.58 \%$) ($\chi^2 = 4.697$, $p = 0.030$). Seed predation under *C. mazapensis* ($6.30 \pm 9.03 \%$) was not significantly different than in open areas ($\chi^2 = 3.303$, $p = 0.069$).

3.6 Seedling limitation

3.7 Nurse effect on seedlings survival

Survivorship curves indicated that nurse plant protection was crucial for seedling survival (Long-Rank test $p < 0.0001$) as every seedling found in open areas died during the first month. After 24 months, seedling survival under both nurse plants was close to 5 % (4.98 % 95 % CI 3.75-6.62 under *C. mazapensis* and 4.17 % 95 % CI 3.14-5.55 under *H. speciosa*) (Figure 2).

3.8 Seedling predation

Seedlings survivorship curves were different between and among age and protection conditions (Long-Rank test $p < 0.0001$) such that survival increased with age and protection. By the end of the experiment (24 months), the protected twelve month-old seedlings had the highest survival (91.6 % 95 % CI 87.1-96.4), while the exposed five month-old seedlings had the lowest (0.91 %, 95 % CI 0.441-1.91). Survival of the exposed seedlings was generally low, with the exception of the twelve month-old seedlings (44.1 %, 95 % CI 36.1-51.8), which was not statistically different from the protected ten month-old seedlings ($p = 0.8574$) (Figure 3).

4 Discussion

The amount of seeds produced by *Cephalocereus polylophus* (976 ± 80 seeds per fruit (Arroyo-Cosultchi et al., 2016)) and their high viability suggest a high potential for recruitment. However, seedling establishment is clearly limited by the removal of the seeds, the dependence on nurse plants, and the low survival rate of seedlings by both abiotic (high PAR) and biotic factors (predation).

Most of the seeds of *C. polylophus* are viable after three years of storage and have high germination rates under laboratory conditions. Seed viability is known to vary strongly in Cactaceae (Rojas-Aréchiga et al., 2013), the seeds of some species can remain viable for one or two years whereas others are viable for more than ten years after their collection (Fearn, 1977; Flores-Martínez et al., 2009; Rojas-Aréchiga et al., 2013). The potential longevity of seeds (viability under laboratory conditions) together with other morphological and physiological traits, such as seed size, photoblastism and secondary dormancy have been used as indicators of the potential to form soil seed banks (Baskin and Baskin, 1989; Rojas-Aréchiga and Batis, 2001; Flores-Martínez et al., 2009). So far, the study of seed soil banks in cacti is limited and few studies have evaluated the distribution, density and viability of seeds in nature (Álvarez-Espino et al., 2014). Considering that *C. polylophus* seeds are neutral photoblastic (Rojas-Aréchiga, 2014) and the ecological longevity (viability in nature) is expected to differ from the potential longevity (Álvarez-Espino et al., 2014; Ordonez-Salanueva et al., 2017), there is a need to evaluate seed viability in natural conditions and over long time periods, such that if seeds can remain viable in nature for more than a year it would suggest a potential to form a short-term persistent seed bank (Thompson et al., 1993; Mandujano et al., 1998; Clark et al., 2007; Ordonez-Salanueva et al., 2017; Lindow-López et al., 2018).

Several factors diminish seed availability. The rapid removal of seeds from the soil reduces the number of seeds available for germination for several cacti species (Turner et al., 1966; Méndez et al., 2006; Miranda-Jácome et al., 2013; Landero and Valiente-Banuet, 2010). In some cases less than 5 % of the total seeds released in every reproductive season escape granivory and remain available for germination (Turner et al., 1966; Bowers, 2000; Mandujano et al., 2001). In *C. polylophus* seed removal was close to 60 % mainly carried out by ants regardless of whether they were

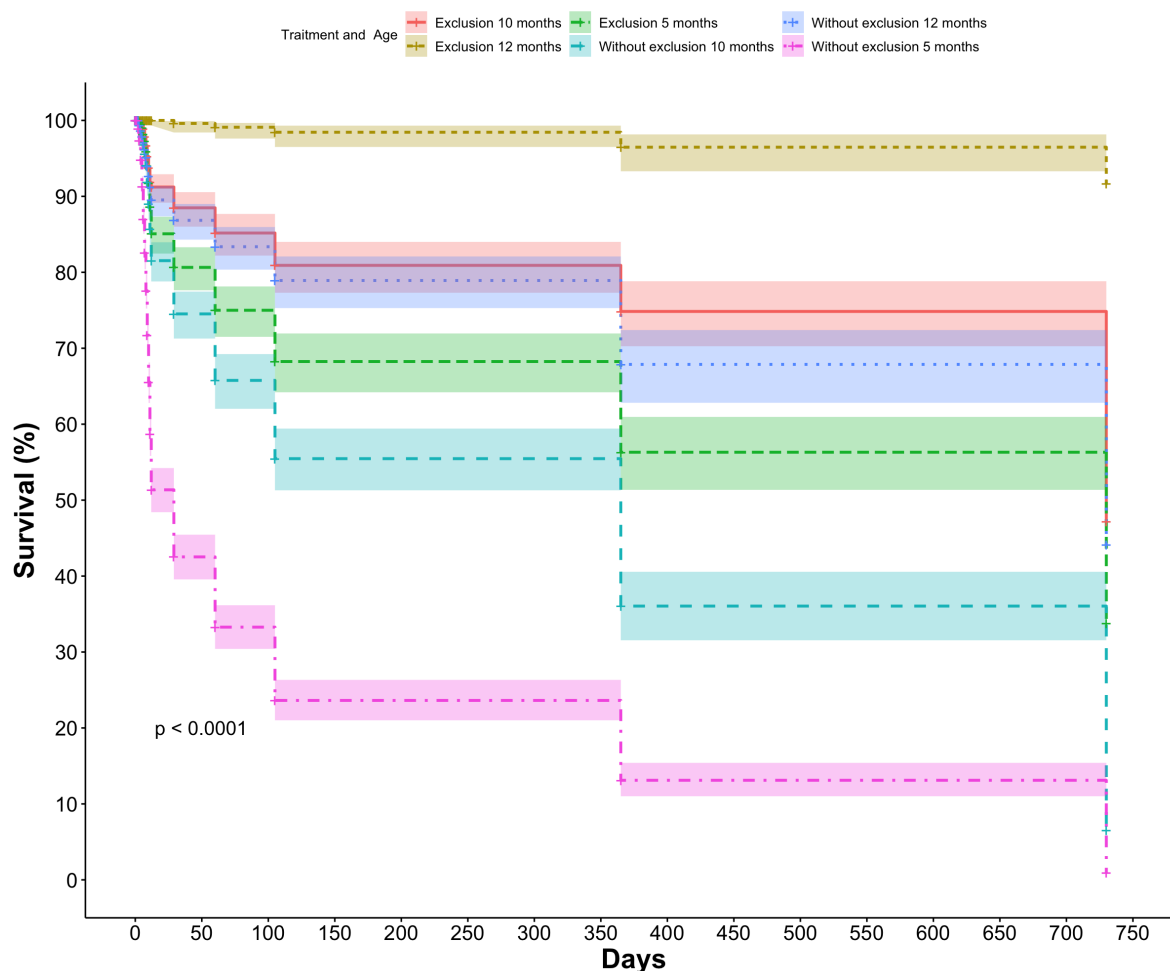


Fig. 3 Survival curves of *Cephalocereus polylophus* seedlings of different age and under different exclusion treatments estimated using Kaplan-Meier's method during a two-year period (August 2014 - August 2016) at Barranca de Metztitlán Biosphere Reserve, Hidalgo, Mexico. Confidence intervals at 95 % (width of the curve). Overlapped curves indicate non-statistical differences between treatments, $n_{initial}=100$ seedlings per treatment.

414 protected or exposed. Seed removal was also high beneath
 415 the canopy of the nurse plants, but we can not confirm that
 416 all the removed seeds were predated, some might have been
 417 abandoned intact during the transport to the nest and remain
 418 available for germination (Vander et al., 2005; García-Chávez
 419 et al., 2010). However, as observed for other cacti (Montiel
 420 and Montaña, 2003; García-Chávez et al., 2010), the removal
 421 of seeds located in favorable microhabitats has a strong dem-
 422 ographic impact since these seeds are the most likely to
 423 germinate (Chambers and McMahon, 1994).

424 *Croton mazapensis* was the shrub that filtered the most
 425 PAR and provided seeds with a more suitable micro-habitat.
 426 From the results of PAR experiment and the fact that *C. poly-*
 427 *lophus* is neutral photoblastic (Rojas-Aréchiga et al., 2013),
 428 we can conclude that low PAR favors germination, although,
 429 poor light also induced the etiolation (the excessive elonga-
 430 tion of the shoot) of seedlings. The dependency on nurse
 431 plants for germination together with the variable weather in

the Metztitlán Biosphere Reserve might explain the contrast-
 432 ing recruitment rates observed during dry (9 recruits / 140
 433 m^2) and wet years (127 recruits / 140 m^2) (Arroyo-Cosultchi
 434 et al., 2016). The survival of the *C. polylophus* seedlings
 435 associated with nurse plants and the null seedling survival in
 436 open spaces during the first month, highlights facilitation as
 437 a crucial association for seedling establishment and is consis-
 438 tent with the pattern reported for other cacti (Turner et al.,
 439 1966; Jordan and Nobel, 1979; Valiente-Banuet and Ezcurra,
 440 1991) and other members of *Cephalocereus* (Landerio and
 441 Valiente-Banuet, 2010).
 442

The observed survival of *C. polylophus* seedlings reveals
 443 a pattern in which survival increases with age. The protection
 444 against herbivores had a positive effect on seedling survival
 445 and the combination both older seedlings and exclusion of
 446 herbivores resulted in close to 100 % seedling survival. Her-
 447 bivory is a factor that negatively impacts many species of
 448 cacti, specially during the earlier stages of their life cycle
 449

(Turner et al., 1966; Mcauliffe, 1984; Mandujano et al., 1998; Landero and Valiente-Banuet, 2010). As with other cacti species, it is clear that the age of the seedlings and its associated increment in size allowed seedlings to escape predation (Steenbergh and Lowe, 1977; Valiente-Banuet and Ezcurra, 1991; Munguía-Rosas and Sosa, 2008). Additionally, larger seedlings are capable of storing a larger volume of water, which enables them to face extended droughts (Jordan and Nobel, 1981), and, although we did not measure root mass, it is likely that the largest seedlings also had greater root volume and root surface, which improved water and nutrient uptake (Tsakalimi et al., 2012).

In addition to the severe bottlenecks for seeds and seedling in natural conditions (Mandujano et al., 1996; Clark et al., 2007; Holland and Molina-Freaner, 2012), the habitats of many cacti are subject to different degrees of anthropogenic disturbance (Martorell and Peters, 2005; Ureta and Martorell, 2009). Activities such as branch cutting and extensive cattle grazing constitute a threat to cacti given their dependency on nurse plants during establishment (Martorell et al., 2015). Habitat conservation is therefore crucial for nurse-dependent species such as *C. polylophus* as the success of seedling establishment is largely affected by the size, density and complexity of their nurse species (Bruno et al., 2003; Aranda-Pineda et al., 2021).

5 Conclusion

Conservation efforts are usually orientated towards increasing population growth rate (Ureta and Martorell, 2009). This can be achieved by acting upon the demographic processes with strong effects on population dynamics (Silvertown et al., 1996; Ureta and Martorell, 2009; Flores-Martínez et al., 2010). Simulations of vital rates suggest that in most cases, seedling limitation has a larger effect than seed limitation in population dynamics of columnar cacti (Arroyo-Cosultchi. Pers com), therefore manipulations to reduce seedling limitations through the introduction of nursery reared seedlings are a better management strategy than sowing seeds directly into the wild (Birnbaum et al., 2011; Reemts et al., 2014). Future restoration and conservation efforts of *C. polylophus* and other columnar cacti should focus on increasing the availability of safe sites for recruitment and seedling augmentation through the introduction of mature seedlings into habitat and the conservation of native vegetation.

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Conflict of interest. The authors declare that they have no conflict of interest.

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Figures

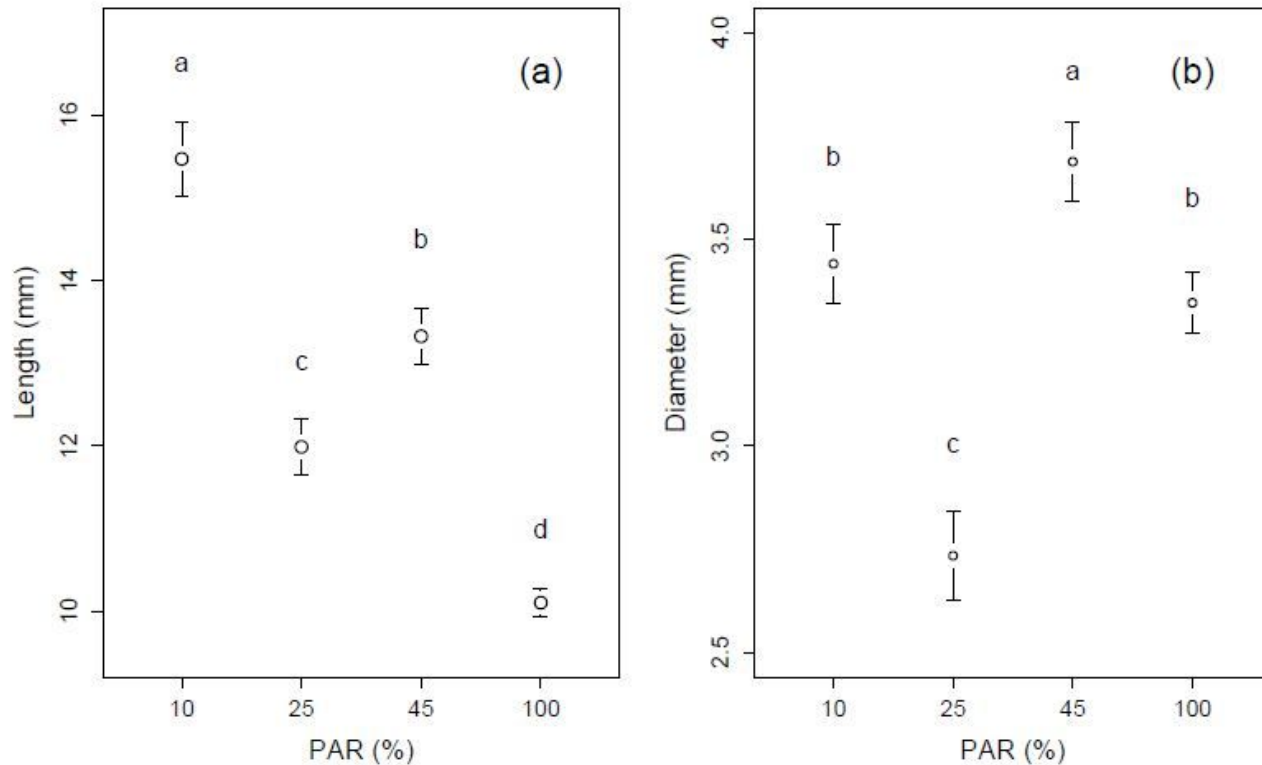


Figure 1

Length (a) and diameter (b) (Mean ± confidence interval 95 %) of *Cephalocereus polylophus* seedlings germinated under different Photosynthetically Active Radiation (PAR) intensities. Different letters indicate differences among PAR treatments for the response variable ($p < 0.05$).

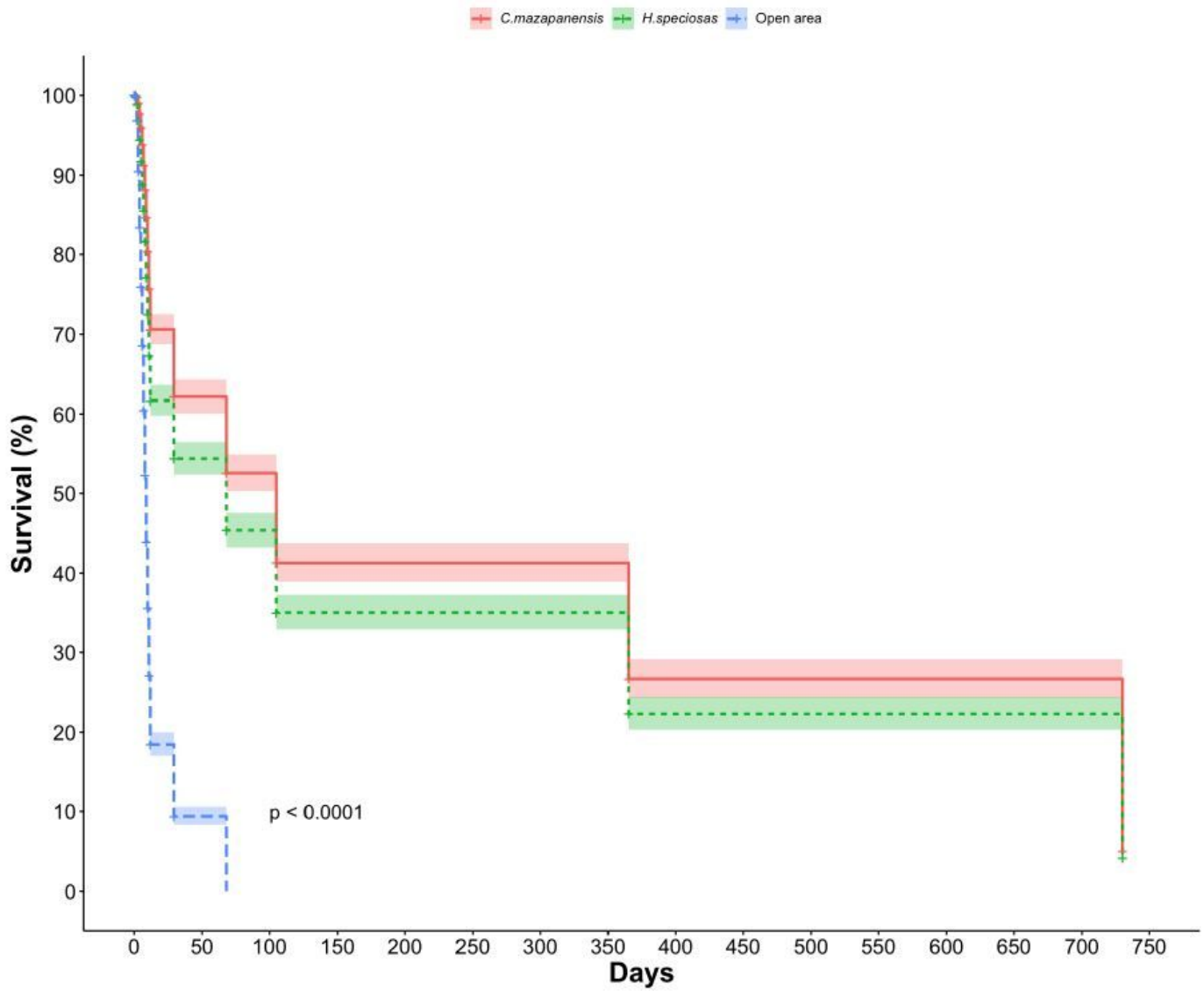


Figure 2

Survival of 10 month-old *Cephalocereus polylophus* seedlings transplanted under *Croton mazapanensis*, *Hoverdenia speciosa* and open areas during a two year period (August 2014 - August 2016) at Barranca de Metzti'an Biosphere Reserve, Hidalgo, Mexico using Kaplan-Meier's method. Confidence intervals at 95 % (width of the curve), ninitial=230 seedlings per condition.

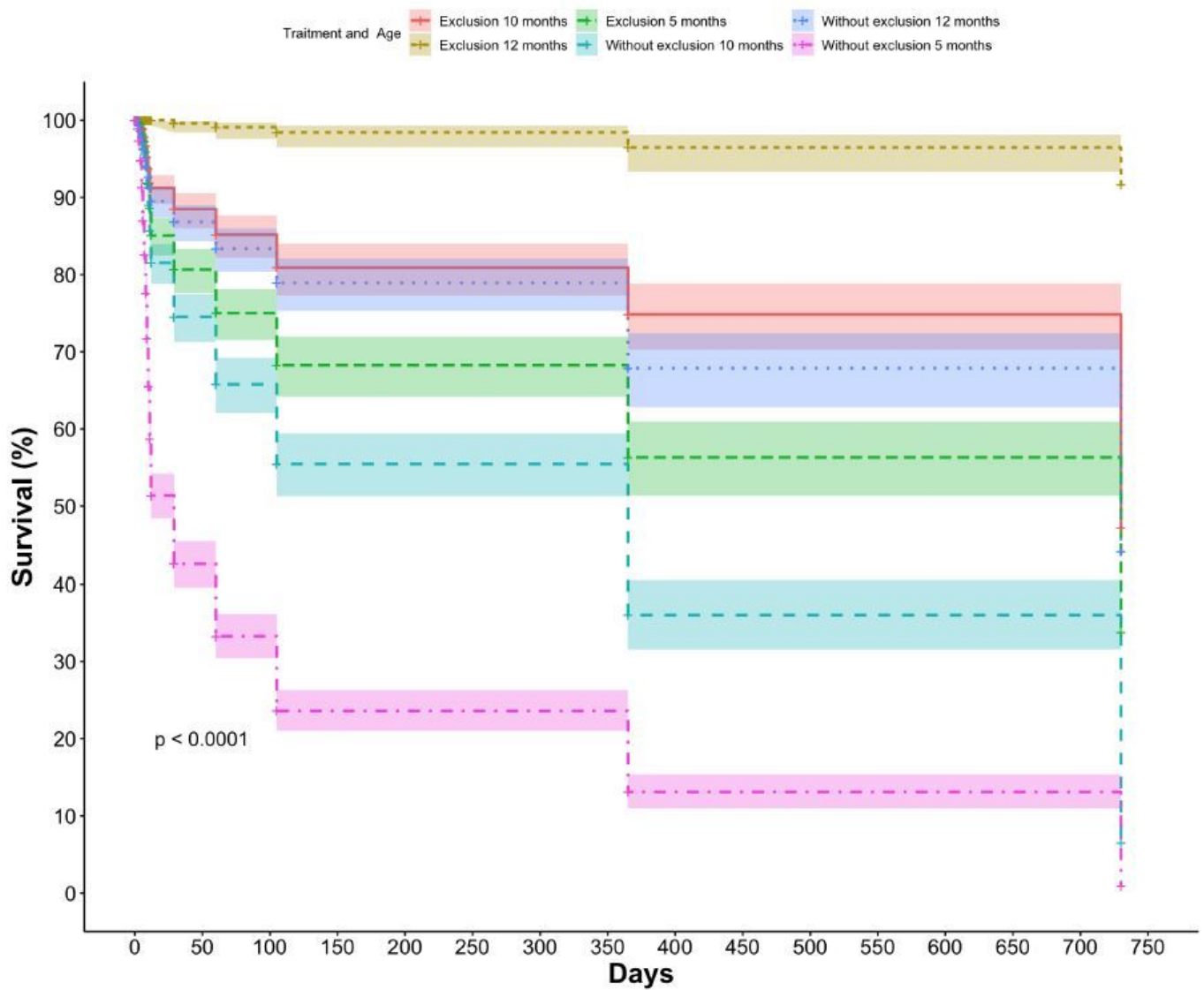


Figure 3

Survival curves of *Cephalocereus polylophus* seedlings of different age and under different exclusion treatments estimated using Kaplan-Meier's method during a two-year period (August 2014 - August 2016) at Barranca de Metztil'an Biosphere Reserve, Hidalgo, Mexico. Confidence intervals at 95 % (width of the curve). Overlapped curves indicate non-statistical differences between treatments, n initial=100 seedlings per treatment.